Archives of Biochemistry and Biophysics 552–553 (2014) 117–127

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

# Random myosin loss along thick-filaments increases myosin attachment time and the proportion of bound myosin heads to mitigate force decline in skeletal muscle

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ARTICLE INFO

*Article history:*
Received 24 September 2013
and in revised form 19 December 2013
Available online 31 January 2014

*Keywords:*
Muscle mechanics
Sarcomere structure
Thick-filaments
Computational modeling
Cross-bridge kinetics

ABSTRACT

Diminished skeletal muscle performance with aging, disuse, and disease may be partially attributed to the loss of myofilament proteins. Several laboratories have found a disproportionate loss of myosin protein content relative to other myofilament proteins, but due to methodological limitations, the structural manifestation of this protein loss is unknown. To investigate how variations in myosin content affect ensemble cross-bridge behavior and force production we simulated muscle contraction in the half-sarcomere as myosin was removed either (i) uniformly, from the *Z*-line end of thick-filaments, or (ii) randomly, along the length of thick-filaments. Uniform myosin removal decreased force production, showing a slightly steeper force-to-myosin content relationship than the 1:1 relationship that would be expected from the loss of cross-bridges. Random myosin removal also decreased force production, but this decrease was less than observed with uniform myosin loss, largely due to increased myosin attachment time ($t_{on}$) and fractional cross-bridge binding with random myosin loss. These findings support our prior observations that prolonged $t_{on}$ may augment force production in single fibers with randomly reduced myosin content from chronic heart failure patients. These simulations also illustrate that the pattern of myosin loss along thick-filaments influences ensemble cross-bridge behavior and maintenance of force throughout the sarcomere.



## Introduction

Several physiological and pathological conditions (*e.g.* aging, muscle disuse, heart disease, lung disease, and cancer) are associated with reductions in skeletal muscle size and function, which contribute to the development of physical disability [12]. Reductions in myofilament protein content and/or function are potential effectors for these tissue-level changes, as these comprise 80% of muscle fiber volume [20] and generate the molecular forces that drive muscle contraction. Interestingly, recent studies have suggested that quantitative alterations in myofilament proteins may not be stoichiometric; more specifically, data from animal models and clinical populations have described a phenomenon of preferential depletion of the contractile protein myosin from muscle tissue/fibers relative to tissue/fiber size or volume [1,11,19,24,26,32,41]. Notably, the loss of myosin is functionally manifest as reduced contractile force per muscle size [11,15,26], presumably due to a reduction in the number of available cross-bridges. In this context, a selective loss of myosin may partially explain the reductions in whole muscle function in numerous physiological and pathological conditions that disproportionately exceed the loss of muscle size [2,16,39,40].

Nearly all of the studies characterizing a selective loss of myosin from skeletal muscle have utilized biochemical measurements [1,11,19,26,32,41], although our previous work also provided mechanical evidence for a loss of myosin [31]. An immediate question that arises from these observations is: how does the selective loss of myosin from the myofilaments manifest itself structurally? Early evidence supported a wholesale loss of thick-filaments from the myofilament lattice [24] and a complete dissolution of sarcomeric structure [26]. These changes, however, were described in conditions of surgical or pharmacological ablation of motor drive to the muscle via dennervation and neuromuscular blocking agents, which are more severe than most acute/chronic physiological or pathological conditions that lead to muscle dysfunction and disability. As an example, our recent findings in human patients

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http://dx.doi.org/10.1016/j.abb.2014.01.015
0003-9861/

with chronic heart failure show a similar preferential loss of myosin, but no gross ultrastructural changes in myofilament fractional content, thick-to-thin filament stoichiometry, or thick-filament length [31]. By default, we concluded that myosin was lost at random along the length of the thick-filament, in keeping with the notion that thick-filaments are remodeled along their length via removal and addition of myosin molecules [13,42]. Anatomical evidence to support this conclusion, however, is beyond current methodological capabilities.

Computational modeling offers an opportunity to address biological questions in complex integrative systems, where current experimental methodologies may have a limited capacity to interrogate coordinated, multi-scale mechanisms underlying physiological function. As introduced above, variation in myosin content in muscle cells may modulate contraction, likely due to changes in the number or kinetic properties of bound cross-bridges. Similarly, contractility can vary with cross-bridge stiffness and thick-to-thin filament overlap at different sarcomere lengths, as these mechanical and structural properties of the myofilament lattice influence the upper limits of cross-bridge binding throughout the sarcomere. To investigate the roles of varied thick-filament structure, sarcomere-length, and cross-bridge stiffness on ensemble cross-bridge binding, force production, and kinetics, we used a computational model of the half-sarcomere to simulate muscle contraction in a system of $Ca^{2+}$-regulated myosin–actin cross-bridge interactions between multiple thick- and thin-filaments [9,37,38]. While cross-bridge stiffness and sarcomere length influenced overall sarcomeric force production by affecting the number and kinetic properties of bound cross-bridges, these measures were influenced more by reductions in myosin content. Specifically, we found that random reductions in myosin content mitigated force decline due to increased $t_{on}$ and fractional cross-bridge binding, which did not occur when myosin content was uniformly reduced from the *Z*-line end of thick-filaments. These observations support our prior speculations that myosin loss occurred randomly in human skeletal muscle fibers of heart failure patients [31], explaining the increase in $t_{on}$ and maintenance of isometric tension [30].

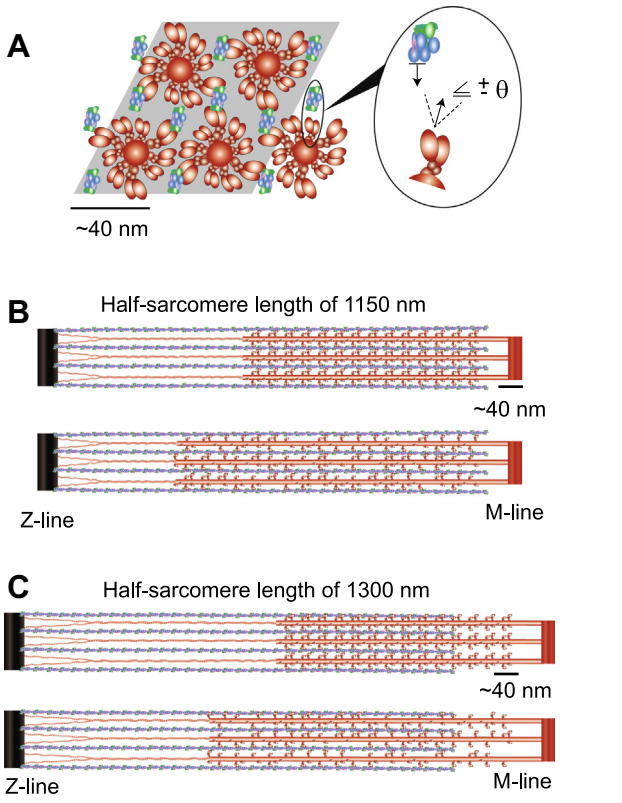


**Fig. 1.** Model geometry and half-sarcomere organization. (A) The shaded parallelogram roughly outlines the cross-sectional view of modeled interactions between four thick-filaments (red) and eight regulated thin-filaments (actin = blue, troponin = green, tropomyosin = magenta) of half-sarcomere length. The expanded inset illustrates one potential myosin–actin cross-bridge interaction between two adjacent filaments. Axial, half-sarcomere illustrations show filament interactions for simulations of (B) 1150 nm (=2.3 μm SL) and (C) 1300 nm (=2.6 μm SL) for uniform (upper) and random (lower) reductions in myosin content at each SL. Titin is represented as the link between thick-filament backbones and the *Z*-line.

## Methods

The computational models used in this work build on a series of spatially-explicit models developed over the past 15 years that simulate muscle contraction within a network of linear springs [9,10,37,38]. In summary, the current model comprises 4 thick-filaments and 8 thin-filaments of half-sarcomere length to model $Ca^{2+}$-regulated actomyosin cross-bridge binding and force production (Fig. 1A). Periodic boundary conditions along the length of the half-sarcomere remove any edge effects along the longitudinal boundary of the simulation, which allows predictions of muscle contraction from a finite number of filaments to represent a subsection myofilament lattice space. Monte Carlo algorithms drive kinetic state transitions for both thin-filament activation and cross-bridge binding. This kinetic scheme includes cooperative activation of thin-filaments from neighboring thin-filament regulatory units and bound cross-bridges [38]. As further described below, we have now: (i) modified myosin organization along thick-filaments to represent the three-start helix of vertebrate thick-filaments (simple-lattice structure [5]), (ii) added a linear elastic element between the free-end of thick-filaments and the *Z*-line to represent titin (Fig. 1B and C), and (iii) developed two different algorithms for altering thick-filament structure to simulate the effect of reduced myosin content on predicted values of force, cross-bridge binding, ATPase, and myosin attachment time ($t_{on}$)[1].

[1] *Abbreviations used:* $t_{on}$, myosin attachment time; SL, sarcomere.

### *Myofilament mechanics and sarcomere architecture*

Thick-filament backbones, thin-filaments, cross-bridges, and titin are represented as linear springs, such that motion, forces, and deformation within the myofilament network occur solely along the axial direction of the filaments (Fig. 1B and C). This affords a linear system of equations to calculate the one dimensional force balance throughout the half-sarcomere at each time-step (=1 ms). These assumptions of linearity allow this system of equations to be solved with linear algebra, which is computationally intensive relative to a mass-action model described by a system of ordinary differential equations [6,33], but less computationally intensive than a multi-dimensional, non-linear force balance that must be solved using optimization algorithms [43–45]. Thick- and thin-filament spring constants were 6060 and 5230 pN $nm^{-1}$ for unstrained spring elements of length 14.3 and 12.3 nm, respectively. Half-sarcomere length thick-filaments were 860 nm (60 thick-filament nodes × 14.3 nm) and thin-filaments were 1110 nm (90 actin nodes × 12.3 nm). These thick-filament and actin node locations coincide with model structures that represent myosin crowns along thick-filament backbones from which myosin heads extend and sites for myosin binding with actin along thin-filaments, respectively.

Three myosin heads extend from each thick-filament node, with each myosin head rotationally spaced 120° apart, such that myosin heads extend from a thick-filament node to face 0°, 120°, and 240°.